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THE ACTIVATION OF GOLD COMPLEXES BY CYANIDE PRODUCED BY POLYMORPHONUCLEAR LEUKOCYTES—I

THE EFFECTS OF AUROCYANIDE ON THE OXIDATIVE BURST OF POLYMORPHONUCLEAR LEUKOCYTES

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Abstract—It has been suggested that the antiarthritic gold complex, aurothiomalate (Autm), is activated by its conversion to aurocyanide by polymorphonuclear leukocytes (PMN) which generate cyanide from thiocyanate. In an examination of this hypothesis, a study has been conducted on the effects of aurocyanide on the oxidative burst of polymorphonuclear leukocytes (PMN) and monocytes activated by phorbol myristate acetate (PMA). Aurocyanide produced delayed inhibition of the oxidative burst as shown by its effect on both lucigenin and luminol-dependent chemiluminescence and on the production of superoxide. It was a more potent inhibitor of luminol-dependent chemiluminescence than free thiomalate and other by-products of the reaction between Autm and cyanide. Aurocyanide had a biphasic effect on the PMA-stimulated hexose monophosphate shunt of PMN, with enhancement at 0.1 μM and inhibition at 10 and 100 μM. The activity of aurocyanide was also compared with that of auranofin, an orally active gold complex, which inhibits a variety of functions of PMN and monocytes. At low concentrations, auranofin produced delayed inhibition of chemiluminescence in a similar fashion to aurocyanide but at high concentrations was an immediate inhibitor of the oxidative burst.

Various gold complexes are used in the treatment of rheumatoid arthritis and juvenile arthritis. The most widely used is sodium aurothiomalate (Autm) which is effective only after parenteral administration. The mechanism of action of Autm has been difficult to study because it generally shows little activity in various cellular systems *in vitro*, particularly in short term experiments and any activity is generally shown only at concentrations which are greater than those achieved during its clinical use [1–4]. The polymeric nature of Autm [5] may limit its cellular uptake and thus its activity *in vitro*. We have suggested that aurothiomalate is activated through interaction with cyanide [6–8] which is produced by activated PMN [9, 10].

Cyanide converts Autm primarily to a mixed complex, $tmAuCN^-$, when one equivalent of cyanide is added and, with the addition of the second equivalent of cyanide, thiomalate (tmH), is displaced and the very stable complex ion, aurocyanide ($Au(CN)_2^-$) is formed [7, 11].

Thus, the principal reactions (ignoring the ionisation of thiomalate) are:

$$Autm + HCN \rightarrow tmAuCN^- + H^+ \quad (1)$$

$$tmAuCN^- + HCN \rightarrow Au(CN)_2^- + tmH. \quad (2)$$

At least three complexes are present at the intermediate stage. Small amounts of both aurocyanide and a bisthiomalate complex, $Au(tm)_2^-$, are present in equilibrium with a marked excess of the mixed complex [7]. The bisthiomalate complex is also formed in aqueous solution by the interaction of equimolar concentrations of Autm and thiomalate [12, 13]:

$$Autm + tmH \rightarrow Au(tm)_2^- + H^+. \quad (3)$$

Aurocyanide is rapidly taken up by red blood cells and it was suggested that this gold complex could enter and affect the function of other cells [6, 7]. Thus, it was hypothesized that Autm is activated by conversion to aurocyanide through the production of cyanide by activated PMN. As a first step in testing this hypothesis, we examined the cellular effects of aurocyanide and other gold complexes which may be formed by the interaction of Autm with cyanide. The functions studied are those associated with the oxidative burst of PMN and monocytes, including the production of superoxide (O_2^-), glucose uptake, the activity of the hexose monophosphate shunt and, particularly, lucigenin- and luminol-dependent chemiluminescence which provide rapid methods of examining the effects of drugs on the oxidative burst. Chemiluminescence with the two probes used is not specific to single products of the oxidative burst but lucigenin is considered to react mainly with O_2^- [14], the first species generated from oxygen during the respiratory burst, while luminol is oxidized mainly by the combination of hydrogen peroxide and hypochlorite [15], which are produced subsequently from O_2^-. In the present studies, superoxide dismutase and azide which is an inhibitor of myeloperoxidase

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[16] were used to determine the major factors responsible for the chemiluminescence of PMN under the conditions used, since superoxide dismutase and azide should have their major effects on lucigenin- and luminol-dependent chemiluminescence [14, 16], respectively.

The activity of aurocyanide has been compared with that of auranofin, a gold complex which, in contrast to Autm, has marked effects on the oxidative burst and other leukocyte functions which have been associated with inflammation [1–4]. While the present studies were not designed primarily to examine the clinical effects of aurocyanide and auranofin, their inhibitory effects on the oxidative burst may have relevance to their clinical efficacy since the oxidative degradation of immunoglobulins by PMN may perpetuate the inflammation of rheumatoid arthritis [17]. Consequently, inhibition of the oxidative burst of PMN may decrease the progression of the disease.

MATERIALS AND METHODS

Materials. The following chemicals and preparations were used: Hank's balanced salt solution (calcium and magnesium free), phosphate buffered saline (calcium and magnesium free), heat inactivated foetal calf serum (all from Flow Laboratories, Sydney, Australia), Ficoll-Paque, Dextran T-500 (Pharmacia AB, Uppsala, Sweden), phorbol myristate acetate (PMA), luminol, lucigenin, type VI cytochrome *c*, superoxide dismutase (bovine erythrocyte), xanthine oxidase (milk) and xanthine (Sigma Chemical Co., St Louis, MO), potassium aurocyanide (Engelhard Ind., Sydney, Australia), sodium aurothiomalate (Autm, May and Baker, Dagenham, U.K.), and auranofin (Smith, Kline and French Laboratories, Sydney, Australia). All other chemicals were of analytical reagent grade. Solutions of potassium cyanide were standardised by titration with silver nitrate [18]. Solutions of Autm were made up freshly in saline before use while stock solutions of PMA (1 mg/mL), luminol (0.1 mg/mL) and auranofin (1 mg/mL) were prepared in dimethyl sulphoxide and stored at 4°.

Subjects. Studies were conducted on the leukocytes of healthy volunteers apart from studies on the PMN of two patients with chronic granulomatous disease whose parents had given informed consent. The study was approved by the ethics committee of the Prince of Wales Hospital.

Cell separation. Heparinized blood (10 mL) was mixed with two volumes of calcium and magnesium free phosphate buffered saline. PMN and mixed mononuclear cells were separated by the method of Boyum [19]. Residual red blood cells in the PMN fraction were removed by lysis in ice-cold water (1 mL) for 30 sec. Both cell fractions were suspended in calcium and magnesium free phosphate buffered saline containing 10 per cent foetal calf serum, centrifuged at 200 *g* for 10 min, washed twice with the same medium, once with a protein-free medium, Krebs–Ringer phosphate containing 5.5 mmol/L glucose together with calcium and magnesium, and finally resuspended in this latter medium. The suspensions of PMN were diluted to a final concentration of 1×10^6/mL while the mixed mononuclear cells were also diluted to contain 1×10^6 monocytes/mL together with a variable number of lymphocytes. After separation, the cells were stored on ice for up to 5 hr.

Oxidative burst. PMN and mononuclear cells (2×10^5 cell/mL) were pre-incubated with the gold complexes and the chemiluminescent probes, luminol (0.6 μg/mL) or lucigenin (0.3 mg/mL), for 10 min at 37°. The cells were then stimulated with PMA (0.1 μg/mL) and the chemiluminescence measured in a Packard Picolite 6500 luminometer modified to maintain the temperature at 37°. The cell suspension was stirred continuously and photon counts were taken over 5 sec intervals every 2 min for 30 min. Experiments were also conducted on the effects of aurocyanide and auranofin on the reduction of cytochrome *c* by PMA-activated PMN. The reduction of cytochrome *c* was measured continuously by the change in absorbance at 550 nm in a Unicam SP1805 spectrophotometer. The major reductant is O_2^- but other products of the oxidative burst also contribute to the reduction of cytochrome *c*. The incubations contained 2×10^5 PMN in 1 mL and the gold complexes were pre-incubated with the PMN for 10 min before the addition of PMA.

Hexose monophosphate shunt. PMN (2×10^6 in 1 mL) were preincubated at 37° for 5 min, where appropriate, with auranofin or aurocyanide, then 0.2 μCi [^{14}C]glucose labelled in the C1 position was added together with either 2 mM methylene blue, which is a stimulator of the hexose monophosphate shunt without stimulating the oxidative burst, or 0.1 μg/mL PMA which leads to enhanced activity of the shunt following the activation of the PMN. After incubation for 1 hr, 0.1 mL hydrochloric acid (2 M) was added, the [^{14}C]CO_2 collected in a solution of 1 M hyamine hydroxide in methanol which was diluted with Pico-Fluor 15 scintillation solution (Packard) and the radioactivity counted in a Packard Tricarb liquid scintillation spectrometer.

Uptake of deoxyglucose. The cellular uptake of deoxyglucose was used as a measure of the uptake of glucose. PMN (2×10^6/mL) were isolated in Krebs–Ringer phosphate containing no glucose and were pre-incubated at 37° for 5 min, where appropriate, with aurocyanide or auranofin, then [^{3}H]deoxyglucose (0.5 μCi, 32 mCi/nmol) was added together with, in some cases, 0.1 μg/mL PMA. After incubation for 60 min, the PMN were washed twice with 10 mL phosphate buffered saline, then mixed with 0.5 mL water and 5 mL Instagel (Packard) and the radioactivity counted as described above.

Cell viability. This was assessed by the exclusion of trypan blue. Cell suspensions were mixed with an equal volume of 0.25% trypan blue in normal saline and the proportion of blue staining cells were determined.

Xanthine oxidase. The scavenging of superoxide by aurocyanide was assessed by its ability to decrease the reduction of cytochrome *c* by xanthine oxidase. Xanthine oxidase (0.04 units/mL), cytochrome *c* (50 μM) and aurocyanide were incubated in Tris buffer (0.1 M, pH 7.4) at 20°, the reaction started by the addition of xanthine (50 μM) and followed by the increase in absorbance at 550 nm.

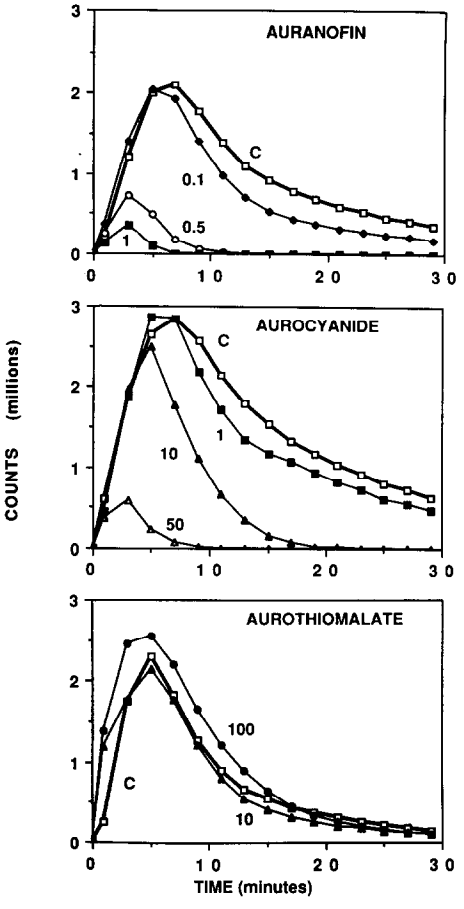

Fig. 1. Typical experiments showing the effects of auranofin (upper). aurocyanide (middle) and aurothiomalate (Autm, lower) on the luminol-dependent chemiluminescence of PMN. PMA was used as the activator of the PMN in all cases. The effects of Autm at concentrations lower than 10 μM were examined but there was no significant alteration in the chemiluminescence of the PMN. Key: control (C, □), 0.1 μM (◆), 0.5 μM (○), 1 μM (■), 10 μM (▲), 50 μM (△), 100 μM (●).

Statistics. The overall significance of treatments was found by analysis of variance with repeated measures while the significance of differences between specific treatments was subsequently determined using Fisher's least significant differences.

RESULTS

Effects of gold complexes on chemiluminescence

Both aurocyanide and auranofin inhibited the chemiluminescence of PMN and monocytes after stimulation by PMA. The effect of aurocyanide followed a characteristic pattern with both types of cells. There was no influence on the chemiluminescence for several minutes but there was subsequent marked inhibition, with the time of commencement of the effect decreasing with increasing concentrations of aurocyanide (Fig. 1). The same pattern of inhibition was seen with both chemiluminescent probes and in both PMN and monocytes. The progressive effect of aurocyanide makes it difficult to quantitate its effect on chemiluminescence, but in order to compare the magnitude of the effect of aurocyanide with that of the other gold complexes, the total chemiluminescence over 0.5 hr was calculated and all the data is presented as a percentage of the control (Fig. 2, Table 1). Aurocyanide inhibited the total chemiluminescence in a dose dependent manner. Inhibition was seen with both probes in both PMN and monocytes. Aurocyanide inhibited the luminol-dependent chemiluminescence of PMN to approximately the same extent as lugicenin-dependent chemiluminescence but, in monocytes, aurocyanide was a somewhat more potent inhibitor of luminol-dependent chemiluminescence (Fig. 2). The data were not sufficient to obtain good estimates of the concentrations of aurocyanide causing 50% inhibition of chemiluminescence (i.e. IC_{50}) in all cases, but the IC_{50} values were approximately 5 μM with both probes in PMN and with the luminol-dependent chemiluminescence of monocytes but about 10 μM with lucigenin-induced chemiluminescence of monocytes (Fig. 2).

Aurocyanide produced a greater degree of suppression of the luminol-dependent chemiluminescence of PMN than the other species involved in the interaction between cyanide and aurothiomalate. Autm had little effect on the chemiluminescence. It increased the chemiluminescence with both probes but significant inhibition was never seen (Fig. 1). Free thiomalate, which is liberated by the reaction between Autm and cyanide was inhibitory only at the highest concentration tested (100 μM, Table 1). The bisthiomalate complex produed a similar effect to that of free thiomalate with significant inhibition of luminol-dependent chemiluminescence only at 100 μM (Table 1). The equimolar mixture of Autm and cyanide produced a dose-related inhibition of the luminol-induced chemiluminescence but was much less potent than preformed aurocyanide (Table 1). The major species in this mixture is the mixed complex, $tmAuCN^-$, but, as discussed previously, small proportions of aurocyanide are present and the observed inhibition could be due to aurocyanide.

The effect of free cyanide on chemiluminescence was quite different to that of aurocyanide. Cyanide had a biphasic effect on the total chemiluminescence in the presence of luminol causing slight but significant inhibition at 10 μM and increased chemiluminescence at 100 μM (Table 1).

The effect of aurocyanide on the oxidative burst of PMN and monocytes was confirmed by its effect on the reduction of cytochrome *c*. As in the chemiluminescence experiments, aurocyanide did not initially affect the reduction of cytochrome *c* and inhibition was produced only some time after stimulation of the respiratory burst. The time at which inhibition commenced decreased with increasing concentrations of aurocyanide (Fig. 3).

Auranofin inhibited the oxidative burst of PMN and monocytes as shown by inhibition of the reduction of cytochrome *c* and chemiluminescence in the presence of both luminol and lucigenin. As was the case with aurocyanide, auranofin inhibited the chemiluminescence of monocytes more potently

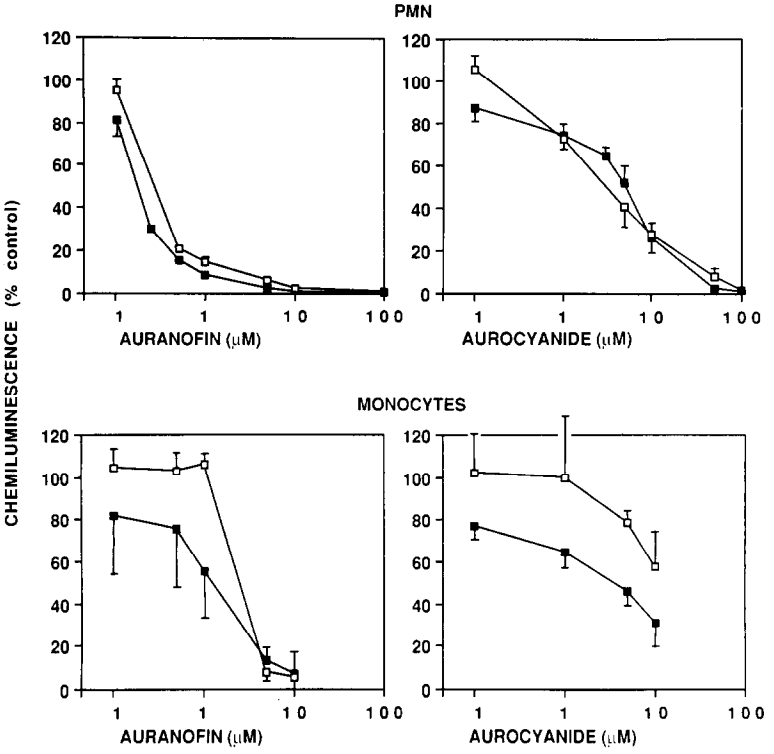

Fig. 2. Influence of varying concentrations of auranofin and aurocyanide on the lucigeninin- (□) and luminol- (■) dependent chemiluminescence of PMN and monocytes (in suspensions of mixed mononuclear cells). Analysis of variance indicates significant effects of the gold complexes of each of the 8 studies ($P < 0.01$). Data are presented as mean ± 95% confidence limits.

Table 1. Effects of Autm, cyanide and products of the reaction between Autm and cyanide on the luminol-dependent chemiluminescence of PMN

	Aurocyanide	Cyanide	$tmAuCN^-$‡	$Au(tm)_2^-$	Thiomalate	Autm
0.1 μM	87 ± 3*					99 ± 3
1 μM	74 ± 3*	119 ± 11	89 ± 6	116 ± 13	101 ± 4	91 ± 3
3 μM	65 ± 5†	143 ± 40	88 ± 6	102 ± 9	106 ± 8	
5 μM	52 ± 2†					91 ± 4
6 μM		98 ± 9	85 ± 4*	94 ± 13	93 ± 7	
10 μM	26 ± 3†	78 ± 4*	72 ± 1†	87 ± 15	87 ± 7	93 ± 6
50 μM	3 ± 2†					
100 μM	0.9 ± 0.2†	140 ± 9*	58 ± 19	47 ± 10†	51 ± 10*	131 ± 4†

Results are presented as percentage of control chemiluminescence counts over 30 min. Means ± SE are shown. Number of independent experiments: 3–11 for aurocyanide, 7 for Autm and 4 for all other species.

Significance levels: * indicates $P < 0.05$ and † indicates $P < 0.01$.

‡ Prepared by mixing solutions of Autm and cyanide in equimolar proportions. The mixed complex, $tmAuCN^-$, is the major species present but small amounts of $Autm_2^-$ and aurocyanide are also present [7].

in the presence of luminol than when lucigenin was used but produced a similar degree of suppression with the two probes in suspensions of PMN. There were, however, some differences between the patterns of inhibition produced by aurocyanide and auranofin. At a low concentration (0.1 μM), auranofin produced late inhibition of chemiluminescence in a similar fashion to aurocyanide, but at higher concentrations, inhibition was immediate on addition of the stimulant, PMA (Fig. 1). The reduction of cytochrome *c* followed a similar pattern with late inhibition at 1 μM auranofin and immediate inhibition at higher concentrations (Fig. 3).

The influence of altered numbers of PMN also showed a contrast between aurocyanide and auranofin. With cell numbers ranging from 1×10^5 to 8×10^5/mL, aurocyanide exerted a relatively constant degree of inhibition of chemiluminescence, whereas the inhibitory effect produced by auranofin decreased markedly with increasing cell numbers

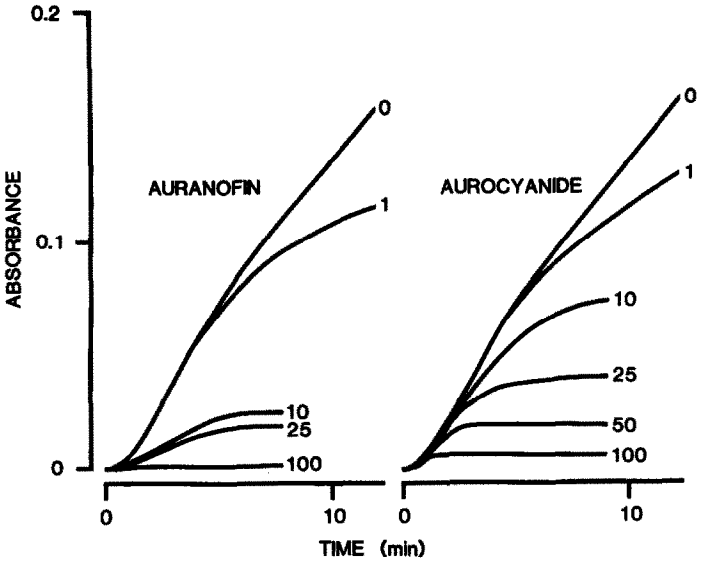

Fig. 3. Typical experiments showing the influence of auranofin (left) and aurocyanide (right) on the reduction of cytochrome *c* by PMN activated by PMA. The major species responsible for the reduction of cytochrome *c* is O_2^-. A change in absorbance of 0.1 is equivalent to the production of 4.74 nmol O_2^-.

(Fig. 4). In the standard method, PMN and monocytes were incubated at a cell density of 2×10^5 cells per mL and thus the effects of auranofin were more marked than at higher cell concentrations.

Specificity of chemiluminescence

Azide (1 mM) inhibited the luminol-dependent chemiluminescence by 88 ± 3% (mean ± SE, N = 5, $P < 0.01$) but had a much lesser effect on lucigenin-dependent chemiluminescence, producing only 29 ± 5% inhibition (N = 7, $P < 0.01$). Superoxide dismutase (0.3 units/mL) inhibited lucigenin-dependent chemiluminescence by 73 ± 4% (N = 6, $P < 0.01$) but was without significant effect on luminol-dependent chemiluminescence (10 ± 3% inhibition, N = 3). These effects are consistent with O_2^- and H_2O_2/hypochlorite being primarily responsible for the chemiluminescence with lucigenin and luminol, respectively [14–16].

Hexose monophosphate shunt

The activity of the hexose monophosphate shunt was estimated by the oxidation of glucose labelled in the C1 position. This does not provide a good measure of the hexose monophosphate shunt in most tissues since all the carbons in glucose can be oxidised. However, PMN contain few mitochondria and the yield of CO_2-^{14}C from glucose-1-^{14}C is much

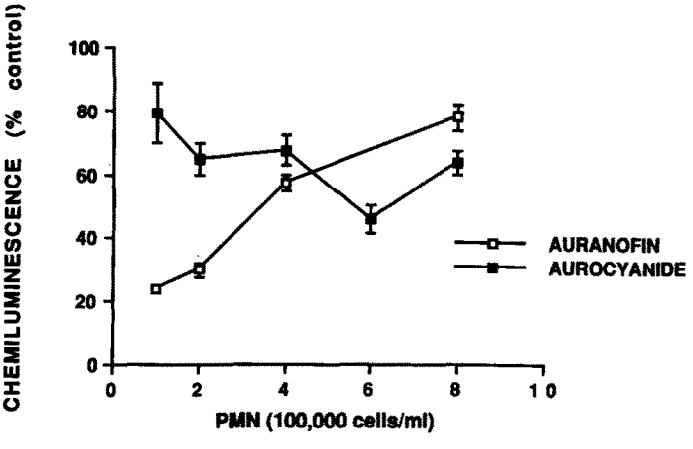

Fig. 4. Effect of auranofin (0.25 μM) and aurocyanide (3 μM) on the luminol-enhanced chemiluminescence of varying cell densities of PMN. Control chemiluminescence (10^6 counts) over 30 min increased in a non-linear fashion from 2.6 ± 0.9, 8.4 ± 1.6, 20.3 ± 2.8 to 49.3 ± 2.1 at 1×10^5, 2×10^5, 4×10^5, 6×10^5 and 8×10^5 PMN/mL, respectively. Data shown as mean ± SE (N = 3–5). Correlation between chemiluminescence (as per cent of control) and cell density was significant with auranofin (r = 0.94, N = 16, $P < 0.01$) but not with aurocyanide.

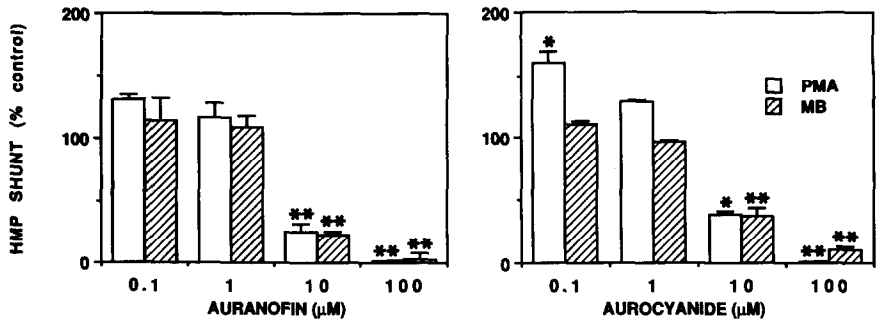

Fig. 5. Effect of auranofin and aurocyanide on the hexose monophosphate activity of PMN stimulated by PMA and methylene blue (MB). Control counts were: 3239 ± 282 cpm and 6185 ± 1200 cpm for methylene blue and PMA stimulated activities, respectively. Data in figure and control values are mean ± SE for methylene blue stimulated activity (N = 3) and mean and range for PMA stimulated hexose monophosphate shunt activity (N = 2). Analysis of variance indicates significant effects of the gold complexes in all experiments ($P < 0.01$). Significance levels of differences from controls are shown as $P < 0.05$ (*) and $P < 0.01$ (**).

higher than when the label is in other positions. For example, the yield of CO_2-^{14}C from glucose-1-^{14}C is five times that from glucose-2-^{14}C [20]. Consequently, C1 labelled glucose is generally considered adequate to estimate the effects of inhibitors on the hexose monophosphate shunt in PMN.

Aurocyanide and auranofin had marked effects on the activity of the hexose monophosphate shunt in PMN. Both complexes had biphasic effects on the shunt when PMA was used as the stimulant. Low concentrations (0.1 and 1 μM) of the gold complexes increased shunt activity while higher concentrations (10 and 100 μM) were inhibitory (Fig. 5). The inhibitory phase was also seen when the hexose monophosphate shunt was stimulated by methylene blue but low concentrations of the gold complexes did not increase shunt activity significantly.

Uptake of deoxyglucose

Both aurocyanide and auranofin influenced the

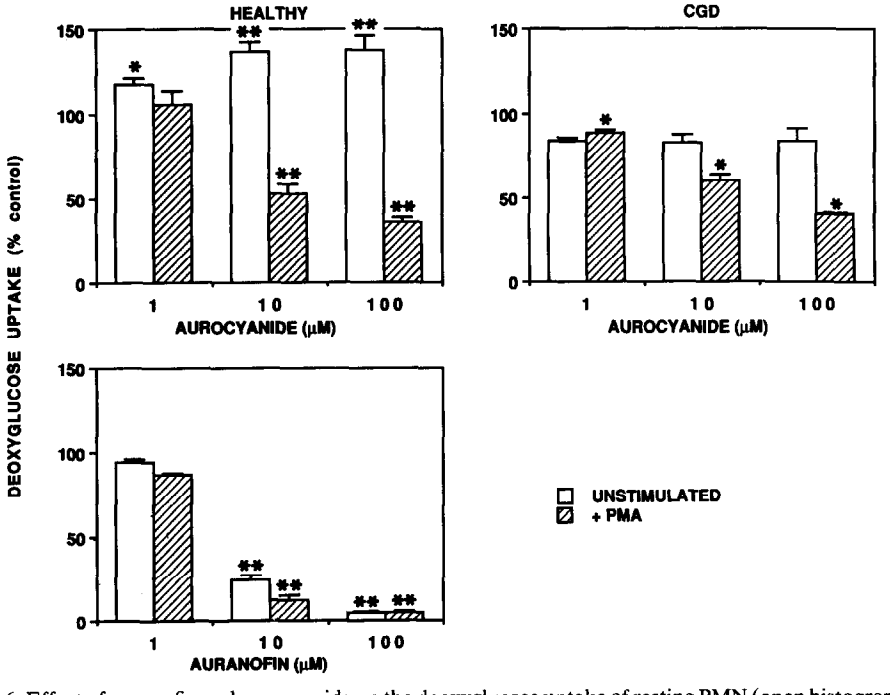

Fig. 6. Effect of auranofin and aurocyanide on the deoxyglucose uptake of resting PMN (open histograms) and PMN stimulated by PMA (shaded histograms). Cells were collected from healthy subjects and patients with chronic granulomatous disease (CGD). Control counts were: 60777 ± 5806 cpm and 47797 ± 5552 cpm (mean ± SE, N = 3) for unstimulated and PMA-activated PMN from healthy patients and were higher at 109052 ± 162 and 114715 ± 5381 (mean and range) for unstimulated and PMA-activated PMN from CGD patients. Analysis of variance indicates significant effects of the gold complexes ($P < 0.01$) in all experiments except in the study of aurocyanide and unstimulated PMN from CGD patients. Significance levels of differences from controls are shown as $P < 0.05$ (*) and $P < 0.01$ (**).

uptake of deoxyglucose by PMN but, again, there was a different pattern of effect of the two gold complexes. Auranofin decreased the uptake of deoxyglucose both by unstimulated cells and cells stimulated by PMA. By contrast, aurocyanide enhanced the uptake of deoxyglucose by unstimulated PMN although there was a significant decrease in the uptake of deoxyglucose by PMN stimulated by PMA (Fig. 6). The influence of aurocyanide on the uptake of deoxyglucose by PMN isolated from patients with chronic granulomatous disease (CGD) provides an interesting contrast with PMN isolated from healthy subjects. In PMN from both CGD patients, the control uptake of deoxyglucose was higher than in PMN from healthy subjects. Aurocyanide inhibited this uptake in the PMA stimulated PMN from these CGD patients but was without significant effect on the unstimulated PMN (Fig. 6).

These effects of the aurocyanide and auranofin on the cellular uptake of deoxyglucose indicate that these gold complexes influence the cellular uptake or phosphorylation of glucose since deoxyglucose is taken up and phosphorylated by cells by the same process as glucose but its further metabolism is poor [21].

Cell viability

Several experiments were conducted to examine the effects of aurocyanide and auranofin on the viability of the PMN. Cell suspensions were examined 10 min after stimulation with PMA. The proportion of cells which were stained with trypan blue was not affected by the presence of 100 μM aurocyanide or auranofin and remained below 2%.

Scavenging

The rate of reduction of cytochrome *c* in the xanthine/xanthine oxidase system was 3.3 ± 0.12 nmol/min (mean ± SE, N = 5). In triplicate experiments in the presence of 5 and 10 μM aurocyanide, the rates of reduction of cytochrome *c* were 101 ± 1 and 102 ± 1% of the mean control value. Thus, aurocyanide, at concentrations which markedly inhibited the oxidative burst of PMN and monocytes, did not inhibit the xanthine/xanthine oxidase system.

DISCUSSION

The present experiments show that aurocyanide is a potent inhibitor of the oxidative burst of both PMN and monocytes. This activity has been confirmed in subsequent work in which evidence for its generation from Autm was also obtained [8]. Aurocyanide is not the only potential product of the reaction of Autm with cyanide produced by activated PMN, but it is a much more potent inhibitor of PMN chemiluminescence than the other products (Table 1). Consequently, aurocyanide is the metabolite of greatest interest at this stage.

The pattern of effects of aurocyanide indicates that the major site of action of aurocyanide on the oxidative burst of PMN does not lie between the first product of the oxidative burst, O_2^-, and the formation of hypochlorite since aurocyanide produced approximately equal inhibition of lucigenin- and luminol-dependent chemiluminescence. By contrast, azide and superoxide, which do affect processes between O_2^- and hypochlorite, produced markedly different effects with the two chemiluminescent probes. In monocytes, both aurocyanide and auranofin were more potent inhibitors of luminol-dependent chemiluminescence and some effect between O_2^- and hypochlorite is possible.

The results exclude several other potential sites of action of aurocyanide on PMN and monocytes. Firstly, the absence of an initial effect on chemiluminescence or on the production of superoxide indicates that aurocyanide does not directly inhibit the NADPH oxidase involved in the production of O_2^-. This lack of initial effect also indicates that aurocyanide is not primarily a scavenger of O_2^-. This was confirmed by its lack of effect in the xanthine/xanthine oxidase system at concentrations which inhibited chemiluminescence and reduction of cytochrome *c* by PMN.

Aurocyanide also had a more potent inhibitory effect on chemiluminescence than on the PMA-stimulated uptake of deoxyglucose and it is unlikely that the effect of aurocyanide on the oxidative burst could be due to inhibition of glucose uptake or phosphorylation. There may still be a common feature in the influence of aurocyanide on glucose uptake and chemiluminescence. The late effect of aurocyanide on chemiluminescence would appear to correspond with its inhibitory effect on the uptake of deoxyglucose being produced in PMA-stimulated but not in resting PMN. However, inhibition of deoxyglucose uptake in activated PMN is not the direct result of the inhibition of the oxidative burst by aurocyanide since the uptake of deoxyglucose was also inhibited not only in PMN from both healthy subjects but also in PMN from patients with chronic granulomatous disease. Cells from these latter patients do not reduce oxygen to free radicals and other products of the oxidative burst of normal PMN and, consequently, the effect of aurocyanide on deoxyglucose uptake cannot be the result of decreased oxidative damage to the PMN.

It is most unlikely that aurocyanide exerts its effects through the liberation of cyanide. Aurocyanide is extremely stable with a formation constant estimated to be 10^{38} [22]. Consequently, very little cyanide should be liberated under physiological conditions and, consistent with this prediction, only a low conversion of aurocyanide to cyanide was detected in a patient following an overdose with aurocyanide [23]. Furthermore, the effects of cyanide on the luminol-dependent chemiluminescence of PMN are quite different to those of aurocyanide (Table 1) while, again in contrast to aurocyanide, cyanide enhances the lucigenin-dependent chemiluminescence and production of O_2^- by PMN [24, 25].

The late effect of aurocyanide on the respiratory burst indicates that aurocyanide may be accelerating mechanisms normally involved in the termination of the respiratory burst. It has been suggested that the availability of reduced nicotinamide adenine phosphate (NADPH) limits the oxidative burst of PMN stimulated by PMA [26]. NADPH is necessary for the generation of superoxide. It is depleted after the

activation of PMN [27] and is regenerated through the activity of the hexose monophosphate shunt. Aurocyanide directly inhibited the activity of the shunt as shown by its inhibitory effect when the shunt was stimulated by methylene blue, but its effect on the activity on the shunt after stimulation of the PMN by PMA was complex. The marked increase in the activity of the hexose monophosphate shunt produced by low concentrations of aurocyanide in PMA-activated PMN contrasts with its inhibition of chemiluminescence and these differing results indicate a dissociation between the effects of aurocyanide on chemiluminescence and on the activity of the hexose monophosphate shunt.

Auranofin shows activity in various cellular systems *in vitro* and the inhibitory activity shown by auranofin in the present studies confirms the results of several previous studies [1–4]. However, the cellular interactions of auranofin and aurocyanide are different. In particular, the contrasting effects of varying cell densities of PMN on the response to auranofin and aurocyanide indicate basic differences in the cellular interactions of the two gold complexes. The most probable explanation for the marked influence of cell density on the response to auranofin is that it is irreversibly bound to PMN. Thus, at any fixed concentration of auranofin, lesser amounts of gold are bound per cell as the cell numbers increase. This conclusion is consistent with the progressive uptake of auranofin by a macrophage-like cell and displacement of the two ligands which bind the gold in the drug [28].

Data presented in this and the subsequent communication [8] provide considerable evidence for the hypothesis that the formation of aurocyanide is responsible for some cellular actions of Autm although much further work is required, particularly from *in vivo* studies, to determine if aurocyanide is responsible either for the characteristic anti-rheumatic activity, such as the slow onset and interpatient variation in response, or the well-known toxic reactions of Autm.

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